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THE THERMAL DEATH POINT IN RELATION TO TIME OF TYPICAL THERMOPHILIC ORGANISMS

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By thermal death point in relation to time is meant the length of time at different temperatures necessary to completely destroy a definite concentration of spores in a medium of known hydrogen-ion concentration.

Several exact methods are in use for determining the death point of various organisms subjected to the influence of moist heat and early investigators have determined with considerable precision the resistance to heat of some of the common micro-organisms. The usual procedure for the determination of the thermal death point of vegetative forms is to expose a suspension in broth or salt solution to the action of a given temperature for 10 minutes. In the case of spores the thermal death point is generally understood as the time necessary to effect the death of all the spores when exposed to 100 C.

Little accurate work has been published on the effect of high temperatures (above 100 C.) on resistant spores, and no reliable method for such determination can be found in the bacteriologic literature. Whenever it has been desired to determine the time above 100 C., varying amounts of calcium chloride have been added to the water in the bath, or the autoclave has been used.

Lawrence and Ford [1] and Laubach, Rice and Ford,[2] in establishing the thermal death points of the spores of some resistant aerobes, subjected suspensions in broth culture to various temperatures both in the Arnold sterilizer and in an autoclave. During their work neither the effect of the time necessary to attain the desired temperature nor of the time allowed for the cooling of the cultures was considered.

The importance, especially in the sterilization of canned foods, of knowing the conditions affecting, and the time necessary for, the complete destruction of resistant spores at high temperatures has led to the introduction of a new method which gives reliable and accurate results for the determination of the thermal death point of heat resis-

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[1] Jour. Bacteriol., 1916, 1, p. 273.

[2] Ibid., p. 493.

tant spores. We give a description of this method together with some results obtained which show not only the effect of different temperatures on the time necessary to destroy a definite suspension of spores of certain typical thermophilic bacteria, but also the influence of different concentrations of the same spores in medium of the same and varying hydrogen-ion concentration.

TECHNIC

Apparatus.—We used the following apparatus: De Khotinsky electric bath with a thermo-regulator attachment; turbine stirrer; motor 1/70 H. P.; liquid of high boiling point; special culture tube—soft glass,[3] closed at one end, 7 mm. (inside diam.) by 250 mm. long with a 1 mm. thickness of wall; Wassermann test-tube rack; wire holders for suspending culture tubes in bath.

The mediums used in this work consist of nutrient agar and broth, prepared according to standard methods, and the juices of the following canned foods: corn, peas, spinach, string beans, pumpkin, sweet potatoes, and beets. These juices are prepared by expressing the contents of the canned food through a flannel cloth by means of a fruit and lard press. The juice is autoclaved at 15 pounds for 5 minutes and the supernatant fluid decanted, tubed and sterilized in the autoclave. This increases the hydrogen-ion concentration of the juice to some extent. The P_H value of some of the juices employed was therefore somewhat lower than is usually found with the corresponding canned foods.

Nutrient agar slants are inoculated with a pure culture of the organism to be tested and grown at the optimum temperature of that specific organism until a luxuriant growth occurs along the inoculated area (24-48 hours). In the case of rapidly growing organisms a shorter period in the incubator will suffice. Usually the growth from two agar slant tubes will produce an adequate number of organisms per cubic centimeter but when dealing with bacteria that grow much less abundantly, as is the case with some thermophiles, 4 to 8 tubes should be used.

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[3] Dr. E. C. Dickson has called the attention of the writers to the fact that the heating in soft glass tubes of unbuffered acid and aqueous solutions, lowers their hydrogen-ion concentration, which does not occur when pyrex glass tubes are used.

The writers find that the various food juices used in the work described in this article remain quite constant in P_H value when heated in soft glass tubes. The buffer substances, presumably, take care of the alkali dissolved from the glass and in soft glass tubes the hydrogen-ion concentration of the food juices does not increase during the heating period as it does in pyrex glass tubes. Better duplicates of the P_H value are secured with pyrex glass than with soft glass tubes.

When the cultures are ready, a smear of each growth is stained and examined microscopically in order to ascertain that pure cultures of the right micro-organisms are obtained. Carefully observing aseptic precaution throughout, a portion of a test tube of sterile nutrient broth, P_H 7 is poured over the surface of the first slant culture, shaking the fluid in such a way as to bring the micro-organisms into suspension. If the culture is not easily washed from the medium, a sterile platinum loop or pipet may be used to remove the growth, care being taken not to cut into the medium and mix the fragments with the bacterial suspension. The suspension thus obtained is poured on the surface of the second culture, bringing this into suspension and repeating the process until the entire series of cultures has been washed, using fresh portions of sterile broth when the suspension has become sufficiently concentrated.

The final bacterial suspension is transferred to a sterile thick-walled flask and incubated for one week at the same temperature as the slants. At the end of the incubation period the inoculated flasks are removed from the incubator and placed in the icebox. After 48 hours they are heated to 85 C. for 15 minutes to kill all vegetative forms, cooled immediately and placed again in the icebox to prevent the germination of spores. This is the stock suspension from which portions are used for the determination of the thermal death point of spores. Nine c c of the medium to be used are inoculated with 1 c c of the stock suspension. This dilution gives satisfactory results and does not appreciably alter the hydrogen-ion concentration of a medium between ranges of P_H 4.5 and 7.0. The special culture tubes described under apparatus, which have been previously wrapped with heavy brown paper in packages of 10 each and sterilized, are inoculated with 1 c c of this suspension of spores. These tubes are of the smallest diameter that can be conveniently used in order to reduce to a minimum the time necessary for the heat to penetrate the center of the medium. They stand a sudden change of temperature over a wide range and can be subjected to the shock of sudden cooling without injury. It has been found that in less than 20 seconds the temperature of the tubes is reduced to that of the ice bath, even when the tubes have been heated at 140 C. These inoculated tubes are then sealed off to within 2 inches of the surface of the liquid, placed in a Wassermann rack and held in a bath of ice water until ready to be heated.

The thermal death point of the spores at 100 C. is determined by immersing the sealed tubes in an open water bath vigorously boiling at 100 C. The De Khotinsky electric bath is used in maintaining a constant temperature, using paraffin oil or a hydrogenated oil for temperatures of 105 to 140 C. The adjustment to the desired temperature is effected by means of a thermoregulator attachment and the temperature kept uniform throughout the bath by means of a turbine stirrer operated by a $\frac{1}{70}$ H. P. motor. A thermometer graduated to 0.1 C., standardized by the Bureau of Standards, is kept at a constant level in the oil to record the temperature. In our experiments the temperature of the oil bath did not vary more than 0.1 + or — after calculating for the stem correction at all temperatures. For temperatures of 105 C. to 125 C., inclusive, the sealed tubes are immersed in a bath of boiling water for 15 seconds before being subjected to the oil bath adjusted at the desired temperature. When a temperature above 125 C. is used, the cultures are exposed in an intermediary oil bath of 115 to 120 C. for 15 seconds. When it is desired to determine the time necessary to destroy a definite number of spores at temperatures at or below boiling, no preliminary heating is given.

Before immersing the sealed tubes in this adjusted bath, the temperature is increased one half of one degree in order to compensate for the loss in the temperature due to the immersion of the tubes in the oil. During the 15 seconds which are allowed for the heat to reach the center of the tubes, before recording the time, the temperature of the oil bath due to the cooling effect of the tubes drops to the temperature at which it was previously adjusted and thereafter remains constant.

A series of tubes, each containing the same concentration of spores, is exposed to the desired temperature for definite periods of time. At the end of each period a tube is removed and immediately placed in a bath of ice water in order to prevent further action of the heat on the spores of the bacteria. When cold, these tubes are placed in an icebox and held until the sterility of the medium can be determined.

Sterility is determined by inoculating nutrient broth with the contents of the heated tubes and incubating for one week. Growth is confirmed by making streak plates from the nutrient broth cultures. In most cases, when spores have survived the heating, growth occurs within 48 hours, and in no case has growth occurred after the fourth day. Some workers have found that the germination of spores which have been subjected to high temperatures is delayed for unusually long periods of incubation. Burke[4] reported that spores of B. botulinus in brain medium survived heating in the autoclave at 15 pounds pressure for 10 minutes, the growth in subcultures being determined only after 53 days' incubation. Experiments in connection with this work carried out according to the method outlined above gives evidence of no retarded germination of spores of typical thermophilic organisms heated at high temperatures, and tubes showing no growth after one week's incubation are considered sterile.

The initial concentration of the spores is determined according to standard methods, the plates being incubated for 48 hours. The hydrogen-ion concentration of the medium is determined colorimetrically. Electrometric measurements made to check the colorimetric method showed close agreement in every case. The determination of the entire series is made on the same day so as to obtain constant and comparable condition.

EXPERIMENTAL

The method described was used to determine the relationship between time and temperature necessary for the complete destruction of the spores of some typical thermophilic bacteria. The temperatures were 100 to 140 C. at intervals of 5 C., and the medium was the juice pressed from canned corn, P_H 6.1. The same concentration of spores which had been subjected to the same preliminary treatment was used at each temperature, and all the conditions standardized as far as possible. The organisms were true thermophiles isolated from cases of spoilage in a variety of canned foods. The presence of these organisms in the canned product without exception was due to their great heat resistance and their ability to withstand the usual sterilizing processes.

Although these obligate thermophiles were found to be the most heat-resistant of all the organisms studied, their power of heat resistance varied greatly for the different species, as can be readily seen from table 1. The range in resistance of those tested varied from 3 to 23 minutes at 120 C. Spores of the same organism varied in resistance to heat under special conditions and could be increased greatly. For example, one type was increased from 12 to 23 minutes at 120 C. within 6 months through repeated artificial cultivation. The same increase in resistance was accomplished by cultivating the surviving spores after understerilizing a suspension. By careful selection, a very resistant type was produced. The retention of stock suspensions for 6 months in the icebox did not alter appreciably the resistance of the spores.

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[4] Jour. Am. Med. Assn., 1919, 72, p. 88.

The results of several experiments over a lapse of several months under different conditions shows that the time necessary to destroy completely a definite concentration of spores is greatly reduced as the temperature is increased.

Table 1 gives the time in minutes necessary to destroy a known concentration of spores of some typical thermophilic bacteria at temperatures from 100 to 140 C., giving in the left hand column the

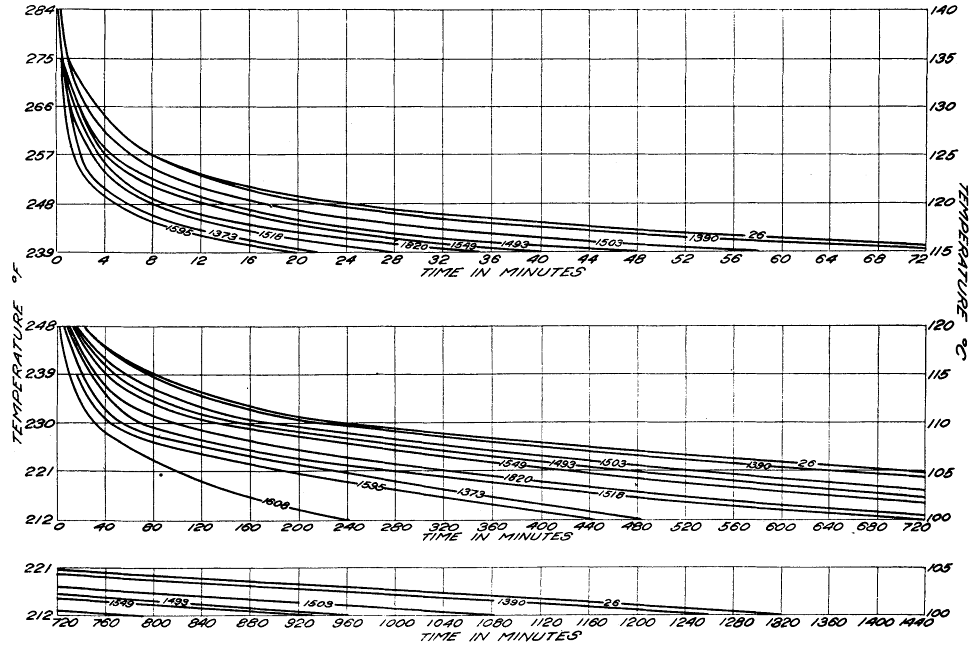


Fig. 1.—Thermal death point curves of typical thermophilic organisms, PH 6.

longest time during which growth occurred and in the right hand column the first time growth did not occur, showing the destruction of all the spores present.

Figure 1 shows the effect of the temperature on the sterilizing time as represented by curves. Each curve is designated by the laboratory number of the organism with which it was obtained. It will be noted that the curves are approximately parallel so that a knowledge of the time necessary to destroy 50,000 spores of an organism of this class at a given temperature enables us to construct the entire curve.

TABLE 1

Time Necessary to Destroy the Spores of Typical Thermophiles at Temperatures Stated

Culture Number	Number of Bacterial Spores per C c	Corn Juice P_H 6.1	Minutes Required to Destroy Spores in Corn Juice P_H 6.1 at																	
			100 C.		105 C.		110 C.		115 C.		120 C.		125 C.		130 C.		135 C.		140 C.	
			+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
1608	12,000	6.1	210	240	95	100	30	32	4	5	2	3	...	1						*
1356	85,000	6.1	300	360	130	150	32	35	12	15	3	4	1	1.25	...	0.75				
1595	100,000	6.1	420	450	220	225	45	48	18	20	4	5	1	1.5	0.5	0.75	....	0.5		*
1373	6,500	6.1	450	480	225	235	50	53	22	24	5	6	1.5	2	1	1.25	0.5	0.66	....	0.5 *
1518	25,000	6.1	660	720	250	270	60	70	25	30	7	8	2	3	1	1.25	0.5	0.66		*
1820	25,000	6.1	720	750	300	360	90	95	30	35	8	9	3	3.5	1	1.5	0.5	0.66		*
1476	70,000	6.1	750	780	380	400	110	115	33	36	9	10	3.5	4	1.5	1.75	0.5	0.66	....	0.5
1492	225,000	6.1	780	840	360	390	100	105	35	38	10	11	3.5	4	1.5	1.75	0.5	0.66		
1549	50,000	6.1	900	930	400	420	130	135	35	40	12	13	3.5	4	1.5	2.0	0.66	0.75		*
1493	4,500	6.1	930	960	450	480	145	150	45	50	13	14	4	5	1.5	2.0	0.75	1.0	....	0.5 *
1503	100,000	6.1	1020	1080	500	525	170	175	55	58	16	17	5	6	2.0	2.5	0.75	1.0	....	0.5 *
4109	12,000	6.1	1080	1140	550	575	180	185	70	72	17	18	6	7	2.5	3.0	0.75	1.0	0.5	0.75
1592	115,000	6.1	1140	1200	575	600	185	190	66	70	18	19	6	7	2.5	3.0	0.75	1.0	0.5	0.75
1390	35,000	6.1	1200	1260	630	660	210	215	75	78	21	22	7	8	3.0	3.5	1.0	1.25	0.75	1.0 *
26	200,000	6.1	1260	1320	660	690	220	225	80	84	22	23	7	8	3.0	3.5	1.0	1.25	0.75	1.0 *

* Organisms used for Chart 1.

The thermal death point of the spores of resistant organisms is influenced by such factors as hydrogen-ion concentration, initial concentration of spores, age and the condition of spore and heat penetration. We shall consider the first two factors.

INFLUENCE OF HYDROGEN-ION CONCENTRATION

The influence of the hydrogen-ion concentration on the thermal death point of spores is shown in table 2 and figure 1. This was obtained by heating a number of spores at a constant temperature in food juices of different P_H values. The work thus far has been done solely on food juices, owing to the necessity of obtaining immediate results to assist the commercial canner in the processing of canned foods. This phase of the problem is to be continued by varying the hydrogen-ion concentration in the same medium. The results so far indicate, however, that the hydrogen-ion concentration of the medium inoculated with a suspension of spores is most important, and that the time necessary for the destruction of bacterial spores is largely dependent on this factor. It is believed that the time necessary to destroy a definite number of spores in string bean juice or corn juice will be nearly the same as that in any solution with the same P_H value, provided the other influencing factors remain constant. Table 2 shows the time necessary to destroy a specified number of spores in the following juices: corn, peas, sweet potatoes, pumpkin, spinach, string beans and beets, when heated at 120, 115, 110 and 100 C. The suspensions for the same organisms were treated similarly at the four temperatures, using the same initial concentration of spores in each case. The results are given in the same way as in table 1, showing the longest time at which growth occurred and the first time at which no growth occurred. The hydrogen-ion concentration of each juice employed as determined electrometrically, is as follows: corn 6.1, peas 5.3, sweet potato 5.0, spinach 5.0, string beans 5.0, beets 4.7, and pumpkin 4.5.

Figures 2, 3, and 4 give curves showing the relation of time to hydrogen-ion concentration of representative thermophilic organisms. In Figures 3 and 4 it will be noted that three distinct results were obtained at P_H 5. This is explained by the fact that three of the juices employed (sweet potato, spinach and string beans) had a P_H value of 5. It is obvious from this that hydrogen-ion concentration is not the only important factor that has a bearing on thermal death point. Notwithstanding this, the curves show a marked relationship between the hydrogen-ion concentration and thermal death point.

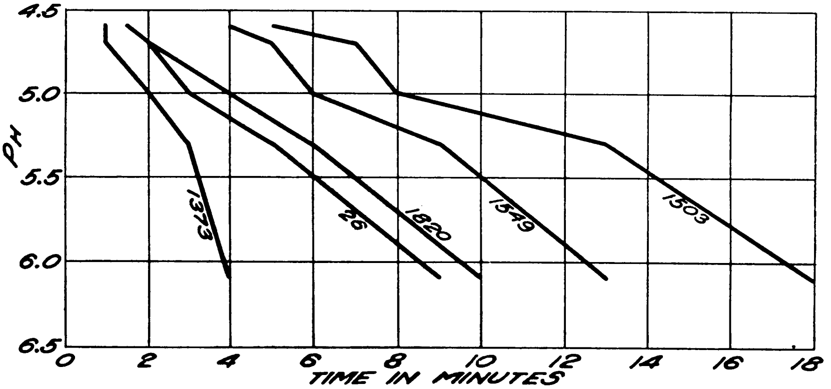


Fig. 2.—Influence of hydrogen-ion concentration of various food juices on the time necessary to destroy spores heated at 120 C.

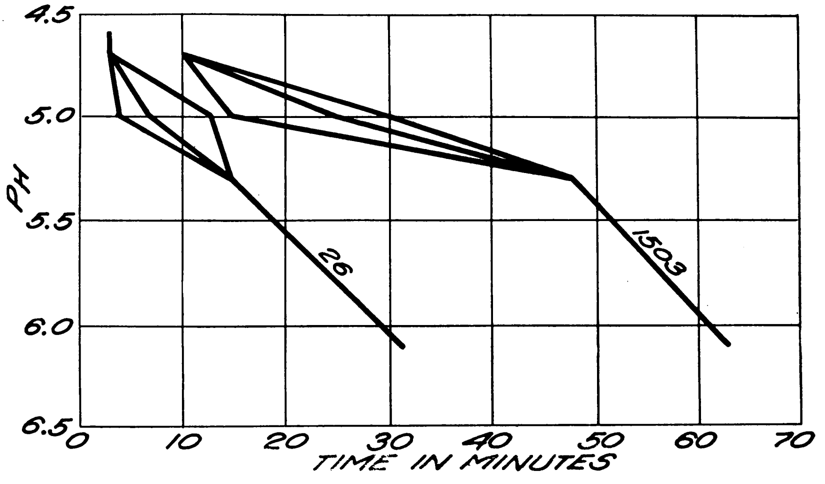


Fig. 3.—Influence of hydrogen-ion concentration of various food juices on the time necessary to destroy spores heated at 115 C.

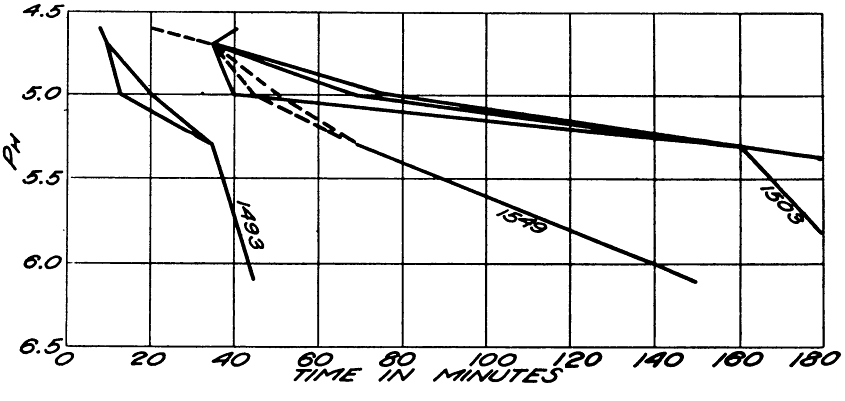


Fig. 4.—Influence of hydrogen-ion concentration of various food juices on the time necessary to destroy spores heated at 110 C.

TABLE 2

THERMAL DEATH POINT OF THE SPORES OF TYPICAL THERMOPHILES IN MEDIUMS OF DIFFERENT HYDROGEN-ION CONCENTRATION WHEN HEATED AT TEMPERATURES STATED

	Culture Number	Number of Spores per C c	Minutes Required to Detroy Spores Heated in													
			Corn Juice PH 6.1		Pea Juice PH 5.3		Sweet Potato Juice PH 5.0		Spinach Juice PH 5.0		String Bean Juice PH 5.0		Beet Juice PH 4.7		Pumpkin Juice PH 4.5	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−
120 C.	1503	150,000	17	18	12	13	7	8	6	7	7	8	6	7	4	5
	4109	40,000	16	17	9	10	9	10	4	5	6	7	4	5	4	5
	1390	60,000	13	14	9	10	7	8	3	4	3	4	4	5	3	4
	1549	50,000	12	13	8	9	5	6	3	4	3	4	4	5	2	3
	1492	10,000	11	12	4	5	2	3	2	3	2	3	2	3	2	3
	1476	100,000	9	10	4	5	2	3	1	2	2	3	1	2	2	3
	1820	100,000	9	10	5	6	3	4	3	4	3	4	1	2	1	2
	26	100,000	8	9	4	5	2	3	2	3	2	3	1	2	1	2
	1518	10,000	7	8	6	7	2	3	4	5	4	5	2	3	4	5
	1356	85,000	5	6	4	5	2	3	2	3	2	3	1	2	1	2
	1508	500,000	5	6	3	4	1	2	1	2	1	2	1	2	1	2
	1493	150,000	4	5	3	4	1	2	1	2	1	2	0.5	1	1	2
	1373	100,000	3	4	2	3	1	2	1	2	1	2	0.5	1	0.5	1
	1608	25,000	2	3	2	3	1	1.5	1	1.5	1	1.5	0.3	0.5	1	1.5
115 C.	1503	150,000	60	63	45	48	28	30	13	15	20	25	9	10	10	10.5
	4109	40,000	58	60	40	45	25	28	9	10	15	18	9	12	10	11
	1390	60,000	55	58	35	40	25	28	6	7	6	7	10	12	6	7
	1549	50,000	35	40	25	30	15	18	10	12	10	12	12	15	5	6
	1492	10,000	30	35	12	15	9	10	6	7	6	7	4	5	4	5
	1476	100,000	33	35	8	10	5	6	2	3	5	6	2	3	5	6
	1820	100,000	30	32	15	18	10	11	9	10	9	10	3	4	9	10
	26	100,000	28	31	12	15	11	13	3	4	5	7	2	3	2	3
	1518	10,000	20	25	20	25	12	13	10	12	10	12	12	13	10	12
	1356	85,000	12	15	10	12	6	8	3	4	3	4	3	4	3	4
	1508	500,000	12	15	10	12	4	5	2	3	2	3	2	3	2	3
	1493	150,000	18	20	10	12	6	7	3	4	3	4	2	3	2	3
	1373	100,000	12	15	7	8	4	5	3	4	3	4	2	3	1	2
	1608	25,000	4	5	4	5	2	2.5	1.5	2.0	1.5	2.0	1	1.5	1.5	2
110 C.	1503	150,000	180	190	150	160	70	75	35	40	65	70	30	35	35	40
	4109	40,000	160	165	90	100	70	75	28	30	28	30	25	28	28	30
	1390	60,000	130	140	90	95	65	70	20	25	20	25	25	28	20	25
	1549	50,000	140	145	60	70	45	50	40	45	40	45	30	35	18	20
	1493	10,000	110	120	40	45	20	25	20	25	20	25	12	15	12	15
	1476	100,000	110	120	35	40	12	15	4	5	12	14	4	5	12	14
	1820	100,000	110	120	70	75	30	35	25	30	25	28	15	18	25	28
	26	100,000	110	120	60	65	26	30	10	12	12	14	10	12	10	12
	1518	10,000	60	70	60	65	25	30	30	35	30	35	25	30	25	27
	1356	85 000	45	50	40	45	20	25	15	20	15	20	15	20	10	12
	1508	500,000	35	40	30	35	4	5	4	5	4	5	4	5	4	5
	1493	150,000	40	45	30	35	15	20	11	13	11	13	8	10	7	8
	1373	100,000	30	35	20	25	5	6	4	5	4	5	2	3	4	5
	1608	25,000	30	32	25	30	3	4	2	3	2	3	2	3	2	3
100 C.	1503	150,000	1140	1200	960	1020	300	360	180	210	300	360	180	210	180	210
	4109	40,000	1140	1200	600	720	300	360	150	180	150	180	180	210	150	180
	1390	60,000	900	960	800	840	...	...	240	270	240	270	210	240	240	270
	1549	50,000	900	960	720	780	...	...	120	180	180	240	180	210	60	90
	1492	10,000	840	900	600	720	...	...	180	210	180	210	120	150	120	150
	1476	100,000	720	840	540	600	...	...	30	60	120	150	30	40	120	150
	1820	100,000	1200	1320	720	840	...	...	180	240	180	240	150	180	180	210
	26	100,000	840	900	540	600	270	300	180	210	150	180	120	150	120	150
	1518	10,000	720	740	690	720	120	150	150	180	150	180	120	150	120	150
	1356	85,000	420	480	360	420	120	150	120	150	120	150	120	150	90	120
	1508	500,000	300	360	180	240	...	...	60	75	75	90	30	45	60	75
	1493	150,000	480	540	480	510	135	150	120	135	120	135	120	135	45	60
	1373	100,000	300	360	300	360	60	90	45	60	45	60	45	60	45	60
	1608	25,000	180	240	150	180	30	45	25	30	20	25	15	20	25	30

TABLE 3

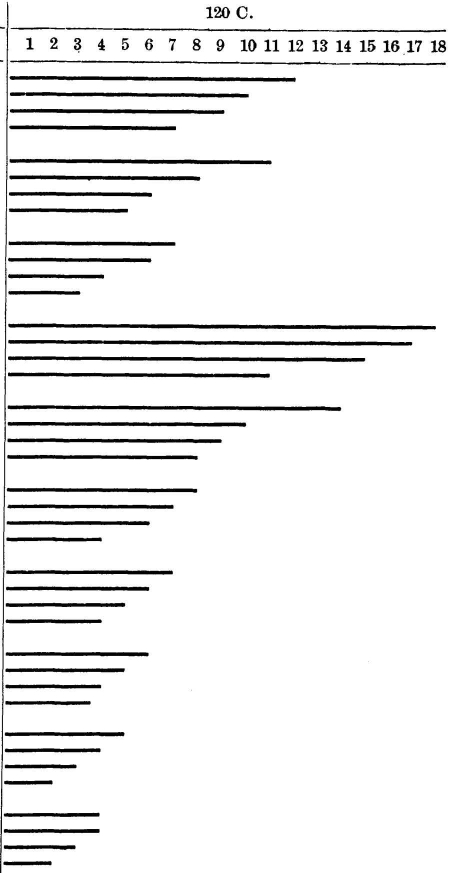
EFFECT OF INITIAL CONCENTRATION ON TIME NECESSARY TO DESTROY THE SPORES OF TYPICAL THERMOPHILES AT TEMPERATURES STATED

Culture Number	Number of Bacterial Spores per C c	P_H Value	Minutes Required to Destroy Spores of Different Concentrations																	
			100 C.		105 C.		110 C.		115 C.		120 C.		125 C.		130 C.		135 C.		140 C.	
			+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
26	200,000	6.0	1260	1320	660	690	220	225	80	84	22	23	7	8	3.0	3.5	1.0	1.25	0.75	1.0
26	20,000	6.0	1080	1140	550	575	180	185	70	72	17	18	6	7	2.5	3.0	0.75	1.0	0.50	0.75
26	2,000	6.0	1020	1080	520	540	170	175	55	60	16	17	5	6	2.0	2.5	0.66	1.0	....	0.66
1592	115,000	6.0	1140	1200	575	600	185	190	66	70	18	19	6	7	2.5	3.0	0.75	1.0	0.5	0.75
1592	10,000	6.0	960	1020	480	495	155	160	48	50	15	16	4	5	2.0	2.5	0.75	1.0	....	0.66
1493	175,000	6.0	1020	1080	500	525	170	175	55	58	16	17	5	6	2.0	2.5	0.75	1.0		
1493	5,000	6.0	900	960	450	480	145	150	45	50	13	14	4	5	1.5	2.0	0.75	1.0	....	0.5

TABLE 4

EFFECT OF INITIAL CONCENTRATION ON TIME NECESSARY TO DESTROY THE SPORES OF TYPICAL THERMOPHILES IN CORN JUICE AT 120 C.

Culture Number	Initial Concentration of Spores	P_H Value	Minutes Required to Destroy Spores of Different Initial Concentrations	
			120 C.	
			+	—
26	40,000	6.0	11	12
	3,800	6.0	9	10
	440	6.0	8	9
	130	6.0	6	7
1421	33,000	6.0	10	11
	3,500	6.0	7	8
	450	6.0	5	6
	80	6.0	4	5
1390	26,000	6.0	6	7
	3,000	6.0	5	6
	400	6.0	3	4
	74	6.0	2	3
4109	130,000	6.0	17	18
	13,000	6.0	16	17
	1,300	6,0	14	15
	130	6.0	10	11
4112	50,000	6.0	13	14
	5,000	6.0	9	10
	500	6.0	8	9
	50	6.0	7	8
1476	120,000	6.0	7	8
	12,000	6.0	6	7
	1,200	6.0	5	6
	120	6.0	3	4
1820	140,000	6.0	6	7
	14,000	6.0	5	6
	1,400	6.0	4	5
	140	6.0	3	4
1356	130,000	6.0	5	6
	13,000	6.0	4	5
	1,800	6.0	3½	4
	130	6.0	3	3½
1493	200,000	6.0	4	5
	20,000	6.0	3	4
	2,000	6.0	2	3
	200	6.0	1	2
1508	350,000	6.0	3	4
	35,000	6.0	3	4
	3,500	6.0	2	3
	350	6.0	1	2



INFLUENCE OF CONCENTRATION OF SPORES

Schmidt,[5] working with spores of the black-leg bacillus, observed that the larger the number of spores the longer the period of heating necessary to destroy them, concluding that the resistance of the spores increases with the number. Gage and Stoughton,[6] in a study of the heat resistance of B. coli, found that the majority of bacteria in a

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[5] Ztschr. f. physik. Chemie., 1906, 21, p. 414.

[6] Science, 1906, 23, p. 216.

TABLE 5

EFFECT OF INITIAL CONCENTRATION ON TIME NECESSARY TO DESTROY THE SPORES OF TYPICAL THERMOPHILES IN CORN JUICE AT TEMPERATURES STATED

Culture Number	Initial Concentration of Spores	PH Value	Minutes Required to Destroy Spores of Different Initial Concentrations					
			120 C.		125 C.		130 C.	
			+	—	+	—	+	—
26	26,500	6.3	18	19	5	6	2.5	3
	2,650	6.3	15	16	3.5	4	2.5	3
1503	40,000	6.3	18	19	5	6	3	3.5
	4,000	6.3	15	16	4	5	2.5	3
1820	130,000	6.3	16	17	5	6	3	3.5
	13,000	6.3	15	16	4	5	2.5	3
1390	85,000	6.3	12	13	4	5	2	2.5
	8,500	6.3	9	10	3	4	2	2.5
1373	4,300	6.3	16	17	5	6	2.5	3
	430	6.3	14	15	4	5	2	2.5
	43	6.3	12	13	4	5	1.5	2
1592	80,000	6.3	14	15	4	5	2	2.5
	8,000	6.3	12	13	4	5	1.5	2
1518	35,000	6.3	8	9	2	3	0.5	1
	3,500	6.3	7	8	1.5	2	1	1.5
1476	15,000	6.3	10	11	3	4	2	2.5
	1,500	6.3	9	10	2	3	1.5	2
	16	6.3	6	7	2	3	1	1.25
1574	40,000	6.3	13	14	4	5	2	3
	4,000	6.3	10	11	4	5	2	2.5
	350	6.3	9	10	3	4	2	2.5
4109	90,000	6.3	16	17	5	6	2.5	3
	9,000	6.3	14	15	4	5	2	2.5
	750	6.3	11	12	3.5	4	2	2.5
	130	6.3	7	8	2	3	1	1.5
1595	380,000	6.3	5	6	3	4	1.5	2
	38,000	6.3	4	5	1.5	2	...	1
	3,500	6.3	3	4	1	1.5	...	0.75
	350	6.3	2	3	...	1		
1321	35,000	6.3	12	13	3	4	1.5	2
	3,500	6.3	10	11	2	3	1	2
	560	6.3	8	9	2	3	1	2
1356	22.500	6.3	16	17	5	6	2.5	3
	2,250	6.3	14	15	4	5	2	3
	456	6.3	12	13	3.5	4	1.5	2
1508	780,000	6.3	3	4	1	1.5	...	0.5
	78,000	6.3	2	3	1	1.5		
1492	50,000	6.3	10	11	3.5	4	1.5	2
	5,000	6.3	8	9	2	3	1.5	2
	700	6.3	7	8	2	3	1	1.5
1493	50,000	6.3	16	17	5	6	2	2.5
	5,000	6.3	11	12	3	4	2	2.5
1549	15,000	6.3	7	8	1.5	2	1	1.5
	1,500	6.3	6	7	2	2.5		
	400	6.3	5	6	1.5	2	0.5	0.75

culture are destroyed by a certain temperature in a relatively short time, while a few individuals will survive much higher temperatures. Eijkman,[7] noticing the variations in rate of death when using different amounts of bacteria, concluded that this was due to substances that were given off into the medium by the killed bacteria.

TABLE 6

EFFECT OF INITIAL CONCENTRATION ON TIME NECESSARY TO DESTROY THE SPORES OF TYPICAL THERMOPHILES IN CORN JUICE AT 125 C. AND 118 C.

Culture Number	Initial Concentration of Spores	P_H Value	Minutes Required to Destroy Spores of Different Initial Concentrations			
			125 C.		118 C.	
			+	−	+	−
26	65,000	6.3	5.5	6		
	22,000	6.3	5	5.5		
	2,260	6.3	5	4.5		
	290	6.3	2	2.5		
1421	27,500	6.3	3	4		
	2,760	6.3	2.5	3		
	500	6.3	2	2.5		
	123	6.3	1.5	2		
1390	43,000	6.3	2	3		
	16,000	6.3	2	2.5		
	2,850	6.3	1.5	2		
	318	6.3	1.5	2		
26	7,000	6.3	...	...	26	29
	1,100	6.3	...	...	21	24
	110	6.3	...	...	16	19
	12	6.3	...	...	5	8
1390	55,000	6.3	...	...	16	19
	14,000	6.3	...	...	16	18
	1,250	6.3	...	...	12	14
	175	6.3	...	...	8	10

The effect of the initial numbers of spores of certain thermophiles on the time required for their complete destruction is determined by diluting the stock culture several times in mediums of the same hydrogen-ion concentration. A series of 9 c c tubes of sterile medium are prepared and dilutions made as follows: 1: 10, 1: 100, 1: 1,000, 1: 10,000, etc., until the final dilution contains less than 10 spores per c c. The thermal death point is determined in the usual manner, and without exception the result shows that the larger the number of spores present in the medium, the longer the time necessary to destroy them. Table 2 gives the results as obtained with different initial concentrations in corn juice, P_H 6, at temperatures from 100 to 120 C. Similar suspensions were used at each temperature and the results are comparable in every respect, showing again the relation of time to temperature.

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[7] Biochem. Ztschr., 1908, 11, p. 12.

The remaining tables show the influence of the concentration of spores on the time necessary to destroy the organisms at various temperatures. The results given in the several tables are not comparable with each other as they were obtained with organisms which had been

TABLE 7

EFFECT OF INITIAL CONCENTRATION ON TIME NECESSARY TO DESTROY THE SPORES OF TYPICAL THERMOPHILES IN CORN JUICE AT 115 C., 110 C., 105 C.

Culture Number	Initial Concentration of Spores	P_H Value	Minutes Required to Destroy Spores of Different Initial Concentrations					
			115 C.		110 C.		105 C.	
			+	—	+	—	+	—
26	45,000	6.3	62	65				
	4,300	6.3	30	35				
	400	6.3	25	28				
	40	6.3	20	22				
1390	35,000	6.3	40	42				
	2,550	6.3	24	26				
	275	6.3	19	21				
	58	6.3	8	10				
1421	35,000	6.3	46	50				
	1,000	6.3	26	28				
	100	6.3	15	18				
	13	6.3	8	10				
26	3,700	6.3	34	35				
	610	6.3	30	33				
	61	6.3	23	25				
	3	6.3	15	16				
1390	70,000	6.3	40	42				
	4,500	6.3	28	30				
	600	6.3	22	23				
	65	6.3	11	13				
1421	12,000	6.3	36	39				
	1,500	6.3	27	30				
	123	6.3	18	20				
	18	6.3	10	13				
26	90,000	6.3	...	...	200	210		
	18,500	6.3	...	...	200	205		
	3,200	6.3	.....	...	190	200		
	250	6.3	...	...	170	180		
1390	125,000	6.3	...	...	190	195		
	31,000	6.3	...	...	160	170		
	2,250	6.3	...	...	160	165		
	175	6.3	...	...	150	160		
26	170,000	6.3	...	...	...	...	600	660
	90,000	6.3	...	...	...	...	600	630
	11,500	6.3	...	...	...	...	540	570
	9,000	6.3	...	...	...	...	540	560
	1,100	6.3	...	...	...	...	510	540
	85	6.3	...	...	...	...	480	510
1390	120,000	6.3	...	...	...	...	510	540
	100,000	6.3	...	...	...	...	500	520
	12,000	6.3	...	...	...	...	450	480
	1,100	6.3	...	...	...	...	440	460
	130	6.3	...	...	...	...	400	420
	20	6.3	...	...	...	...	360	390

cultured for various lengths of time and hence were of different resistance.

CONCLUSIONS

A new method for determining the thermal death point of resistant spore-bearing bacteria at high temperature, which gives accurate results, is described.

The time necessary to destroy a known suspension of spores in medium of known hydrogen-ion concentration decreases as the temperature increases. For example, 200,000 spores per c c of culture 26 are destroyed in 1,320 minutes in boiling water (100 C.), 690 minutes at 105 C., 225 minutes at 110 C., 84 minutes at 115 C., 23 minutes at 120 C., 8 minutes at 125 C., 3.5 minutes at 130 C., 1.5 minutes at 135 C., and 1 minute at 140 C. Nineteen thermophilic organisms studied in this connection show the same relationship between time and temperature.

TABLE 8

TIME NECESSARY TO DESTROY SPORES OF TYPICAL THERMOPHILES AT 120 C.

Culture Number	P_H	No. of Bacterial Spores per C c	Minutes Required to Destroy Spores in Corn Juice at 120 C.																													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	28	30	32	36
1608	6.0	12,000	+	⊕	—	—	—	—	..	—	..	—																				
1356	6.0	85,000	..	+	+	—	—	—	—	—	..	—	..	—																		
1595	6.0	100,000	..	+	+	+	—	—	—	—	..	—	..	—																		
1373	6.0	6,500	..	+	..	+	+	—	—	—	..	—	..	—																		
1518	6.0	25,000	..	+	..	+	+	+	⊕	—	—	—	..	—	..	—																
1820	6.0	25,000	..	+	..	+	..	+	+	+	—	—	—	—	..	—																
1476	6.0	70,000	..	..	..	+	..	+	+	+	⊕	—	—	—	..	—																
1492	6.0	225,000	..	..	..	+	..	+	..	+	+	⊕	—	—	—	..	—															
1549	6.0	50,000	..	..	..	..	..	+	..	+	..	+	+	+	—	—	—	—	..	—												
1493	6.0	4,500	..	..	..	..	..	+	..	+	..	+	+	+	+	—	—	—	..	—	..	—										
1503	6.0	100,000	..	..	..	..	..	..	..	+	..	+	..	+	+	..	+	+	—	—	..	—	..	—	..	—						
4109	6.0	12,000	..	..	..	..	..	..	..	..	..	+	..	+	..	+	..	+	+	—	—	—	..	—	..	..	—	..	—			
1592	6.0	115,000	..	..	..	..	..	..	..	..	..	..	..	+	..	..	+	..	⊕	+	—	—	—	—	..	—	..	—	..	—		
1390	6.0	35,000	..	..	..	..	..	..	..	..	..	..	..	+	..	..	+	..	..	+	..	+	+	—	—	—	—	—	..	—	..	—
26	6.0	200,000	..	..	..	..	..	..	..	..	..	..	..	..	..	+	..	+	..	+	..	+	+	+	—	—	..	—	..	—	..	—

+ = growth in 15 hours' incubation at 55 C.
⊕ = growth in 48 hours' incubation at 55 C.
— = no growth in 7 days' incubation at 55 C.

The hydrogen-ion concentration influences the time necessary to destroy a known suspension of spores at a given temperature. As the P_H value is increased, the time required for complete destruction is decreased.

The initial concentration of spores per c c affects the time necessary to sterilize a medium of known hydrogen-ion concentration at a given temperature. The larger the number of spores present in a medium, the longer the time necessary to destroy them.